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Inactivation of *Giardia* Cysts by Chlorine

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Cysts of *Giardia lamblia* from symptomatic and asymptomatic carriers had similar resistances to chlorination.

Waterborne giardiasis has been well established as a cosmopolitan public health problem (4). The efficacy of chlorination for the inactivation of *Giardia lamblia* cysts is a matter of great concern to those responsible for water utility operations in endemic areas. A recent report indicates that viable *G. lamblia* cysts can be destroyed by chlorine (3), but there remains a dearth of information concerning the effects of chlorine on *G. lamblia* cysts from symptomatic and asymptomatic carriers and on other *Giardia* species. This study was undertaken to determine the viability of *G. lamblia* cysts shed from an asymptomatic carrier and to correlate the chlorine resistance of these cysts with those from a symptomatic carrier and another *Giardia* species, *G. muris*, which is infective for mice. The distinctions between symptomatic and asymptomatic carriers described by Wolfe (4) were used in our work.

G. lamblia cysts, present in fecal specimens obtained from a symptomatic donor and an asymptomatic donor, were separated from the fecal material by flotation on 1 M sucrose. Cysts were stored in distilled water at 5°C for a minimum of 1 week to allow for the maturation period described by Bingham et al. (2). The maximum storage time for cysts used in this study was 20 days. Cyst densities were determined by hemacytometer counts. *G. muris* cysts, present in fecal specimens from infected mice, were concentrated by the same procedure.

Stock chlorine used in this experiment was prepared by adding sodium hypochlorite to distilled water. The medium used for exposure of the cysts to chlorine consisted of chlorine demand-free 0.01 M phosphate buffer, adjusted to pH 6.0, 7.0, and 8.0, to which sufficient stock chlorine was added to provide a free residual chlorine concentration of 2.5 mg/liter. All tests were performed at 5°C, a temperature similar to the water temperature encountered in many outbreaks and at which the biocidal efficiency of chlorine is reduced (3). Chlorine determinations were done by using the *N,N*-diethyl-*p*-phenylenediamine colorimetric method (1). To reaction beakers containing 500-ml quantities of demand-free buffer at 5°C, 10^3 cysts per ml were added. Cyst and chlorine controls were included in each experiment. The beakers were immersed in a circulating water bath at 5°C and were stirred mechanically. At appropriate time intervals, chlorine action was stopped by the addition of 1 ml of 10% sodium thiosulfate, the chlorine level having been checked immediately before the addition of the thiosulfate. The cysts were reconcentrated by filtration through a 5.0-μm porosity polycarbonate filter under light vacuum. The cysts were washed from the filters with an aqueous 0.01% Tween 20 solution, using a Pasteur pipette. Cyst viability was determined by a modified in vitro excystation procedure (F. W. Schaefer, E. W. Rice, and J. C. Hoff, Abstr. Annu. Meet. Am. Soc. Parasitol. 1980, abstr. no. 90, p. 51). The number of cysts which excysted was determined by microscopic count. The survival percentages for each experiment were determined by comparing the results of each sample with those of the control excystations, which were arbitrarily adjusted to 100%. Actual control excystations were consistently higher than 90% for the *G. muris* cysts and for the *G. lamblia* cysts from the symptomatic donor. Control excystations of cysts from the asymptomatic donor ranged from 50 to 90%.

The results of duplicate experiments at each pH level for cysts from the symptomatic donor and of triplicate experiments for cysts from the asymptomatic donor and for *G. muris* cysts are shown in Fig. 1. These results showed a somewhat greater resistance for the cysts from the symptomatic donor at short contact times. At longer contact times, the cysts from the symptomatic and asymptomatic donors showed similar levels of viability. The *G. muris* cysts were consistently more resistant than the *G. lamblia* cysts at the maximum exposure times. The data suggest that *G. muris* may be a valid model for *G. lamblia* in disinfection studies, but additional comparative data are needed to substantiate this. As expected, the inactivation of the cysts by chlorine was enhanced at the lower pH

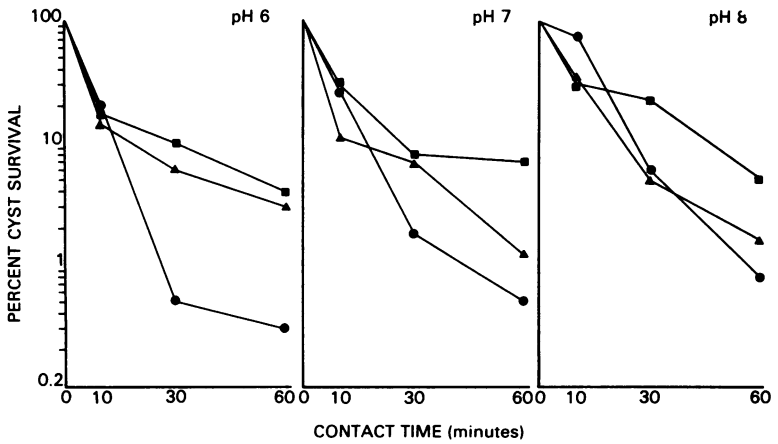

FIG. 1. Representative *Giardia* cyst survival at 5°C in 2.5 mg of chlorine per liter after 10, 30, and 60 min of contact time in buffer at pH 6, 7, and 8. Symbols: (●) *G. lamblia* cysts, symptomatic donor; (▲) *G. lamblia* cysts, asymptomatic donor; (■) *G. muris* cysts.

levels. These findings compared favorably with those of previous investigators (3) and showed that although asymptomatic carriers may shed viable cysts, there was little difference in the chlorine resistance of these cysts compared with that of cysts from a symptomatic carrier.

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